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Clinical Report

Co-infection of Central Nervous System by *M. Tuberculosis*, *Cryptococcus* and possibly *Naegleria fowleri*

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Abstract. Co-infection due to *Mycobacterium tuberculosis*, *Cryptococcus* and *Naegleria fowleri* has not been reported till now in literature, to the best of our knowledge. Here we report a curious case of co-infection of the central nervous system due to these three pathogens in an apparently immune-competent, HIV negative boy. The 15 year old boy was a diagnosed case of tubercular meningitis and was on anti-tubercular and anti-epileptic treatment. However, two months later he presented again in the emergency department with sudden loss of consciousness. His CSF showed presence of capsulated budding yeast cells (suggestive of *Cryptococcus*) and flagellated parasites (resembling the flagellated stage of *Naegleria fowleri*). CSF was also positive for Cryptococcal antigens by Latex Agglutination test. The boy was HIV negative and apparently immuno-competent. He was subsequently put on amphotericin B therapy for six weeks. Repeat microscopy, done towards the end of amphotericin B course, showed clearing of CSF. However, the patient's condition improved only slightly, owing to neurological damage caused by the pathogens as suggested by brain CT and MRI scans. Thus infection caused by the members of three different kingdoms in an apparently immuno-competent boy highlights the importance of thinking beyond the ordinary causative pathogens, and actively searching for rarer etiologies to ensure timely intervention; especially in non-responsive cases.

INTRODUCTION

With almost 40% of the Indian population latently infected with tuberculosis, India is reporting more new TB cases annually than any other country (Central TB Division, Directorate General of Health Services. TB India, 2012). Pulmonary tuberculosis is the most common type, while tubercular meningitis (TBM) is another important entity which is characterized by difficulty in diagnosis and treatment. Cryptococcocal meningitis occurs worldwide, mostly affecting the immunocompromised individuals such as patients with HIV infection, diabetes, cancer, solid organ transplants, haematological malignancies and on chemotherapeutic drugs. Infection in the immuno-competent, though less frequent is also seen. Simultaneous CNS infection with these two organisms has been reported in HIV positive patients (Khan *et al.*, 2009), however it is very rare in HIV negative individuals (Nand *et al.*, 2013).

Naegleria fowleri is a ubiquitous free living amoeba, known to cause Primary amoebic meningoencephalitis (PAM). The disease has a rapid onset with fulminant course. Rapid progression, limited awareness among physicians and lack of definite treatment options makes this a highly fatal condition. Direct microscopy of the CSF

usually shows large amoeboid forms, motile with help of pseudopodia. Pear-shaped bi-flagellated forms are frequently seen in culture, and have also been reported in direct CSF specimens (Sood *et al.*, 2013).

Here we report a case of 15 year old apparently immuno-competent boy who was diagnosed with Tubercular meningitis and Cryptococcal meningitis and had presence of a flagellated parasite in CSF, suggesting co-existence of PAM. To the best of our knowledge, this is the first case of co-infection of these three pathogens.

CASE HISTORY

The patient was a 15 year old boy, apparently healthy two months back when he developed weakness in the right side of the body, associated with fever and vomiting. He went to a local practitioner and took some medication; however the symptoms did not improve. He was brought to our tertiary care hospital hospital, where he was diagnosed as a case of Tubercular meningitis with epileptic episodes, on the basis of *M. tuberculosis* specific PCR of CSF sample. He was put on anti-tubercular treatment (5 drug regimen ATT, i.e. isoniazid, rifampicin, pyrazinamide, ethambutol and streptomycin), along with anti-epileptics and discharged in a satisfactory condition.

One and a half months after discharge, the boy presented again to the emergency department of our tertiary care hospital with sudden loss of consciousness. The patient remained unconscious for 3-4 hours, followed by regaining of consciousness. There was no current history of seizures, headache, trauma or vomiting. On examination, the patient was conscious but had altered sensorium. He was afebrile, blood pressure was 114/72 mm Hg and pulse rate was 78/min. Chest, cardiovascular and per-abdomen examination did not reveal any abnormalities. CNS examination revealed motor weakness in the right upper and lower limbs, with mute right plantar reflex. Bilateral sensations were, however, normal.

Laboratory investigations revealed haemoglobin concentration- 11.7 g/dl, total leukocyte count- 11,000/mm^3 with differential leucocyte count of polymorphs-81, lymphocytes- 16, monocyte- 2. Platelets counts were 3.51 lacs/ mm^3. Blood biochemistry tests were also performed including serum electrolytes (sodium- 135 mmol/l, potassium-4.0 mmol/l, calcium-9.2mmol/l, phosphate- 3.6 mg/dl), liver function tests (total bilirubin- 0.4 mg/dl, AST- 55 u/l, ALT- 12 u/l, serum alkaline phosphatase- 126 U/l, serum protein- 7.4 gm/dl, serum albumin- 3.6 gm/dl, and kidney function tests (blood urea- 47mg/dl, serum creatinine- 0.5mg/dl). The patient was referred to Integrated Counseling and Testing Centre (ICTC) for HIV counseling and testing, where he tested non-reactive. His CD4+ cell count was also within the normal limits (1032 cells/µl). He was however, reactive for Hepatitis B surface antigen.

Chest x-ray showed loculated effusion on the left side, with clear bilateral lung fields. MRI brain revealed an area of altered signal intensity in left fronto-parietal region, likely a late sub-acute infarct with early hydrocephalus. NCCT head showed white matter hypo-density in left frontal white matter, with diffuse cerebral atrophy. The patient was put on Ryle's tube and was managed conservatively in the medicine ward, primarily on ATT and anti-epileptics. The patient, however, did not show much improvement during the course.

One month later, the patient developed jerky bilateral hand movements and labored breathing. Repeat NCCT head showed white matter edema in left parietal lobe with communicating hydrocephalus. Inj. mannitol and syrup glycerol were added to the treatment.

Lumbar puncture was done and CSF sample was collected and sent to the Mycology laboratory for direct microscopic examination and fungal culture. Wet mount preparation with saline showed presence of spherical budding yeast cells and a flagellated parasite. Two to three parasites were present per high power field; they were approximately 15-25 µm in size and appeared to have a single nucleus and two flagella. They were actively motile by continuous beating of flagella. Negative staining with

India ink showed presence of 5-10 budding yeast cells per high power field along with the flagellated parasite. Budding yeast cells were spherical, capsulated, 6-8 µm in size, with morphology consistent with *Cryptococcus*. On direct Gram's staining, the parasite and budding yeast cells were seen. Latex agglutination test was positive for Cryptococcal antigen in CSF. Fungal culture of CSF on Sabouraud's dextrose agar (SDA) and brain heart infusion agar, however, did not show any growth even after 6 weeks of incubation at 25°C and 37°C.

Attempt was made to culture the flagellated parasite on 1.5% non-nutrient agar plates with a lawn culture of *Escherichia coli*. After inoculation, plates were incubated at 37°C and checked microscopically daily (under × 10 and × 40) for clearing of the agar, and presence of parasite for upto 15 days. Unfortunately, areas of clearing/ plaques or parasitic forms were not seen.

On probing, the relatives gave the history of boy bathing in unclean village pond. The patient was started on i.v. amphotericin B (25mg, OD) infusion in 5% dextrose. For approximately one month, the general condition and Glasgow coma scale of the patient waxed and waned. However, after one month of amphotericin B therapy, the patient showed symptomatic improvement. Amphotericin B was continued for another 2 weeks, for a total period of 6 weeks. CSF microscopy, culture and latex agglutination test (for *Cryptococcus*) were repeated, which did not demonstrate any fungal/ parasitic elements. Repeat CSF PCR for *M. tuberculosis* was also negative. MRI was repeated and it showed possible cryptococcoma in left parietal region. The results of drug susceptibility testing of CSF were ready at this stage and revealed resistance of *M. tuberculosis* to isoniazid, following which tab levofloxacin was added to the continuation phase of anti-tubercular therapy.

However, 10 days after the completion of amphotericin B regimen, patient started having fresh episodes seizures, and was loaded with valproate. The patient was subsequently shifted to the neurosurgery Intensive Care Unit. Repeat CSF wet mount, India ink and Gram's stain preparations did not show any capsulated budding yeast cells or flagellated parasites. Fungal and bacterial cultures were also negative. Liver function tests revealed serum AST/ ALT which were raised five times the normal limit, possibly due to ATT induced hepatitis or reactivation of Hepatitis B infection. Anti-tubercular therapy was modified accordingly to ethambutol, streptomycin, levofloxacin, ofloxacin. At the time of this writing, the patient is admitted in the wards, with general condition still being poor. He is on Ryle's tube feed and receiving tab fluconazole (150mg, once daily), continuation phase of ATT, prophylactic anti-epileptics (tab levetiracetam, 500 mg BD, tab sodium valproate, 300mg, OD), tab baclofen 20mg BD, along with sessions of occupational therapy.

DISCUSSION

Tubercular meningitis (TBM) is rare in developed countries with about 100 to 150 cases occurring annually in the US, less than 3% of the estimated 4,100 annual cases of bacterial meningitis (Marx GE, Chan ED, 2011). However, being a tuberculosis endemic nation, TBM is one of the common causes of chronic meningitis in India, though the exact prevalence is not known. Cryptococcal meningitis is the most common presentation of cryptococcosis, with over 1 million cases and 600,000 global deaths per year (Park BJ *et al.*, 2009). Ninety five percent of the Cryptococcosis cases in middle and low income countries have HIV as the risk factor; Infection in apparently immunocompetent being less common (Leal AL *et al.*, 2008).

Tubercular and cryptococcal meningitis are similar in many ways, occurring with increased frequency and severity in patients with immuno-compromised status. Both present as chronic meningitis, persisting for 4 weeks or more (Cohen *et al.*, 2010). In fact, the clinical presentations of the two diseases overlap so closely that the specific diagnosis on the basis of clinical suspicion may not be possible in majority of the cases. Basic CSF characteristics are also frequently similar as both organisms classically produce a

lymphocytic pleocytosis with high CSF protein levels. The diagnosis of either eludes the clinician to such an extent that many times if the diagnosis of one of these conditions is made, the possibility of the other gets sub-consciously ignored. Unfortunately, even if the diagnosis is made accurately, despite prolonged treatment over months or even years, the prognosis remains poor.

The history presented here is of one such rare case, in which an apparently immunocompetent patient was diagnosed with TBM on the basis of CSF TB specific PCR and culture. Despite being started on ATT, he presented again two months later with aggravated symptoms. At this time possibility of another etiology was considered. Lumbar puncture was repeated, and diagnosis of coexisting Cryptococcal meningitis was made on the basis of microscopy and latex agglutination test. However, whether coinfection with *Cryptococcus* was present initially and was missed, or was superimposed later on tubercular meningitis is not known. Previously, similar cases have been documented where the diagnosis of cryptococcal meningitis was missed in favor of TBM (Singh *et al.*, 2013).

This case was made more interesting and unique by visualization of a flagellated parasite in the CSF. Flagellated parasites in CSF are a rare entity, with Trypanosomes being one of important pathogens (Lejon & Bushcher, 2005). However, they are not frequent in India and morphologically they are slender organisms with single flagellum that curves round the body in form of undulating membrane. The parasite seen in the patient's CSF did not resemble a Trypanosome, but it did resemble the flagellated stage of a rarely reported free living amoeba, *Naegleria fowleri* (Figure 1). Unfortunately, the diagnosis could not be confirmed as the parasite did not grow in culture.

The first case of human infection with free living amoebae was described by Fowler & Carter in 1965. Since then close to 440 cases have been reported worldwide, in contrast, from India only 10-15 cases have

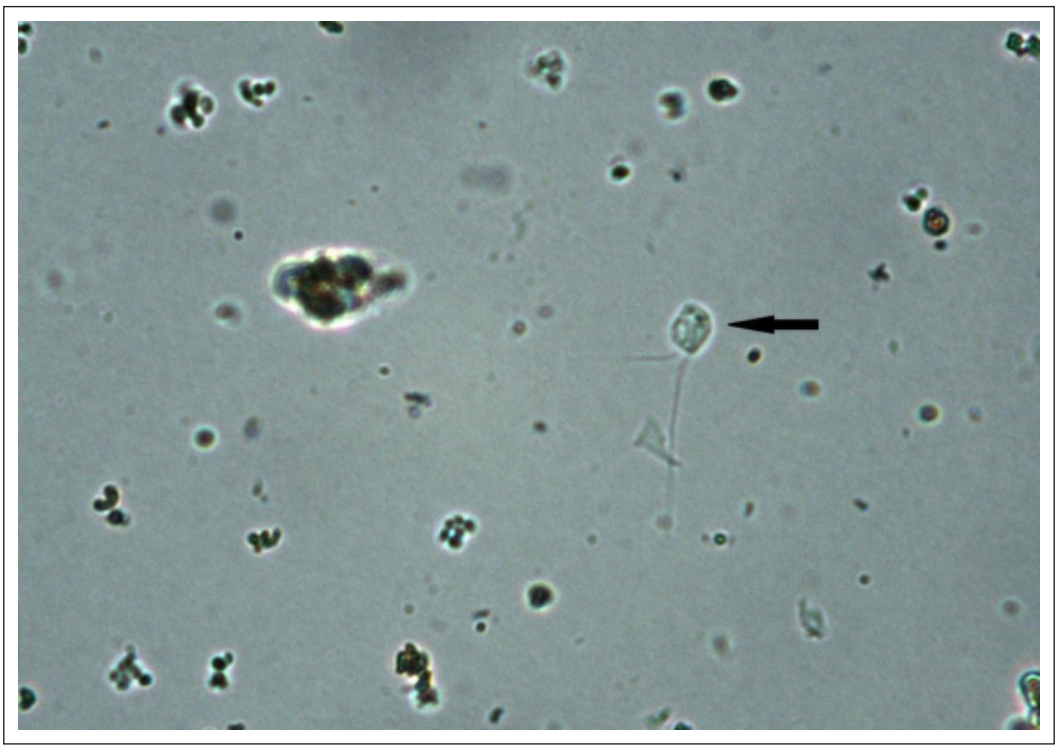

Figure 1. CSF wet mount preparation showing flagellated parasite resembling Naegleria fowleri (1000x)

been reported, probably owing to lack of awareness and poor diagnostic facilities (Sood *et al.*, 2013). *Nagleria fowleri* is found worldwide in moist soil and freshwater. Three morphologic stages exist: the amoeboid form, cyst form and the flagellated stage. The organism enters the nasal cavity when water contaminated with amoebae is aspirated while swimming or bathing in contaminated water. Subsequently, it invades the central nervous system through the olfactory neuroepithelium and causes Primary Amoebic Meningoencephalitits (PAM) infection that clinically resembles acute or hyper-acute meningitis (Visvesvara , 2011). The usual diagnostic form seen in the CSF is the amoeboid form, which changes to the flagellated stage upon culture *in vitro*. However, flagellated forms have reported in the CSF previously.[4] Response to treatment is poor, with most of the cases having fatal outcome, with the associated mortality approaching 98% (Alisky, 2008).

Recommended treatment for PAM is amphotericin B, used either systemically or intrathecally. It may be used in combination with rifampin, fluconazole, sulfadiazine, miconazole, sulfisoxazole, ketoconazole, dexamethasone, ornidazole or chloramphenicol (Sood *et al.*, 2013). In the present case, the patient was administered i.v. amphotericin B, which is effective against both *Naegleria fowleri* and *Cryptococcus*. Repeat CSF examination done after one month was negative for both *Cryptococcus* and the flagellated parasite, suggesting clearing of these two pathogens from the CSF.

However, despite negative CSF reports, the general condition of the patient improved only temporarily, with subsequent exacerbations. These were probably due to neurologic damage caused by multiple microbial assaults; as demonstrated by the radiological reports suggesting late subacute infarct in left fronto-parietal region infarcts, diffuse cerebral atrophy, communicating hydrocephalus and cryptococcomas.

The present case re-emphasizes the importance of clinical acumen in quickly looking for a co-infection in cases of tuberculosis, especially the long-standing unresponsive cases of tubercular meningitis. Three extraordinary pathogens, belonging to three different kingdoms, infecting a single person's single organ system, further makes it is imperative to think beyond the usual etiological agents and look for other unlikely etiologies.

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